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14. ABSTRACT The MMT mice develop multiple mammary carcinomas between the ages of 65-108 days with 100% penetrance. The mammary carcinomas occur in multiple stages. In addition, the progressive malignant transformation is closely correlated with telomerase activity using telomeric repeat amplification protocol (TRAP), suggesting that telomerase may be involved in the development of mammary carcinomas. Furthermore, constitutive deletion of the RNA component of telomerase significantly delayed the appearance of tumor and reduced the tumor burden. In the present project, we aim to assess the immunotherapy of MMT mice with deficiency of telomerase. Vaccination of telomerase heterozygotes MMT mice (GO) and first generation of mTERC-/-MMT mice with fusions of dendritic cells and tumor cells (FC/MUC1) induced CTL activity comparable to that from MMT mice. In addition, the latent time required for tumor development was slight prolonged. Taken together, these results, although preliminary, suggest the feasibility of immunotherapy in the background of telomerase deletion.					
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INTRODUCTION:

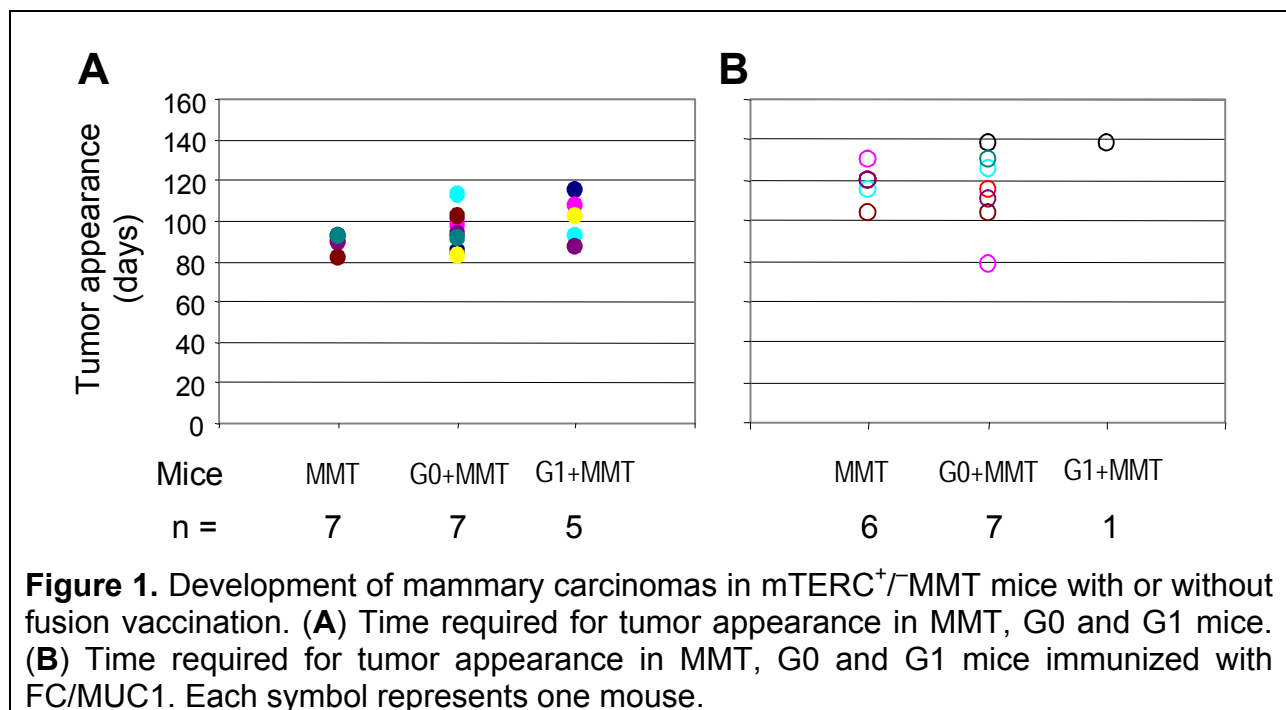
Approximately 90% of human malignant tumors are telomerase-positive. Telomerase is required for full malignant transformation. In our previous study, telomerase activity is closely associated with early and invasive mammary tumors. Collectively, these results suggest that telomerase may be a promising target for cancer therapy. Indeed, inhibition of telomerase by expression of dominant-negative hTERT (1, 2), antisense oligonucleotides (3, 4), or 3'-azido-2',3'-dideoxythymidine (AZT) (5-7) limits the life-span of human cancer cells and results in the death of tumor cells. Most studies, however, have been limited to the use of cultured cells due to lack of an applicable animal model. An *in vivo* model that closely mimics human cancer is desirable for studying the antitelomerase effect on tumor cells as well as on normal tissues. Our initial results show that tumorigenesis of mammary carcinoma is severely compromised by telomerase deficiency. However, tumors still developed in G3 mTERC^{-/-}MMT mice, albeit with much reduced tumor burden. These findings suggest that antitelomerase therapy can inhibit tumor growth but may not be able to eradicate the tumor. Therefore, an additional therapy such as radiation or immunotherapy may be required to achieve elimination of tumor. We aim to use immunotherapy since it has minimal toxicity to the host and works most effectively in the setting of minimal tumor burden. To examine the efficacy of the combined therapies, we have generated successive generations of MMT mice with deficient for telomerase activity. These mice were used to test the efficacy of immunotherapy in the prevention of mammary carcinomas in the background of telomerase deficiency.

BODY:

The proposed study is to explore the combined approaches of telomerase inhibition and immunotherapy in the prevention and treatment of mammary carcinoma. In our previous studies, we have generated MMT mice that develop spontaneous mammary carcinomas. More important, the development of mammary carcinomas is correlated with the telomerase activity. These results indicate that telomerase activity play a role in the formation or progression of mammary tumors, suggesting that telomerase is a potential target in the prevention or treatment of mammary carcinomas. The specific aims of the proposed study remains the same: (i) to determine the effect of telomerase deficiency on both tumorigenesis and the functioning of highly proliferative normal organs; (ii) to determine the synergistic effect in the management of mammary carcinomas by combined immunotherapy and depletion of telomerase activity. In the last eight months since the approval of our animal protocol by DOD, we have generated first and second generations of MMT mice deficient in telomerase activity (mTERC^{-/-} MMT mice). In addition, selected mice were immunized with fusions of dendritic cells (DC) and tumor cells (FC/MUC1). We have obtained preliminary results. In overall, the proposed studies have been executed as planned.

Task 1. To determine the effect of telomerase deficiency on both tumorigenesis and function of highly proliferative normal organs

We have generated MMT mice deficient in telomerase activity in the C57BL/6 strain by crossing mice deficient for RNA component of telomerase (mTERC^{-/-}) with MMT mice. The initial mating resulted in the generation of mTERC heterozygotes (mTERC^{+/-}) carrying PyMT oncogene and/or MUC1 antigen. These mice were mated to generate the first generation (G1) of mTERC-null MMT mice (mTERC^{-/-}MMT). The G1 mice were mated to produce the second generation of mTERC^{-/-}MMT mice (G2). This mating scheme will continue until the fifth generation of mTERC^{-/-}MMT mice. The mean latent time for tumor development in MMT, G0 and G1 mice is 90.17, 95.57 and 100.6 days, respectively (Fig. 1A). There is no statistical significance of latent time for tumor development among the groups. These results are consistent with previous findings that tumor development is not significantly affected in the early generation of telomerase deficiency mice.



Task 2. To determine the synergistic effect in the management of mammary carcinomas by combined immunotherapy and depletion of telomerase activity

Previous studies indicate that deficiency of telomerase activity renders tumor development in MMT mice delayed and tumor burden significantly decreased, thus creating a favorable condition for immunoprevention. To examine whether immunization in the background of telomerase deficiency can enhance the antitumor immunity, MMT (N=6), G0 (N=8), G1 (N=1) and G2 (N=1) mTERC^{-/-}MMT mice were vaccinated subcutaneously with fusions of DC and MC38/MUC1 tumor cells (FC/MUC1). The vaccination was repeated monthly. The mice were examined for the appearance of mammary tumors. Delayed appearance of mammary carcinomas was observed in G1 mTERC^{-/-}MMT mice immunized with FC/MUC1 (Fig. 1B). The time required for the mammary tumors in MMT, G0 and G1 mTERC^{-/-}MMT mice are 118.17±8.5, 114.28±19.86 and 138 days, respectively. One G2 mTERC^{-/-}MMT mice was initially immunized on 9/22/2006 and no mammary tumor was observed yet. The mice were

sacrificed when the mammary tumor reaches 1 cm. At the end of experiment, splenocytes were harvested for the measurement of CTL. As shown in Figure 2, comparable CTL activity against mammary carcinoma cells was observed in MMT, G0 and G1 mTERC^{-/-}MMT mice, suggesting that the cellular immunity is not affected by the deficiency of telomerase activity, at least in the early generations of mTERC^{-/-}MMT mice. Taken together, these results indicate the immunization with FC/MUC1 in early generation of mTERC^{-/-}MMT mice can induce CTL activity comparable to that in MMT mice. In addition, the latent time for tumor development was prolonged in immunized G1 mTERC^{-/-}MMT mice.

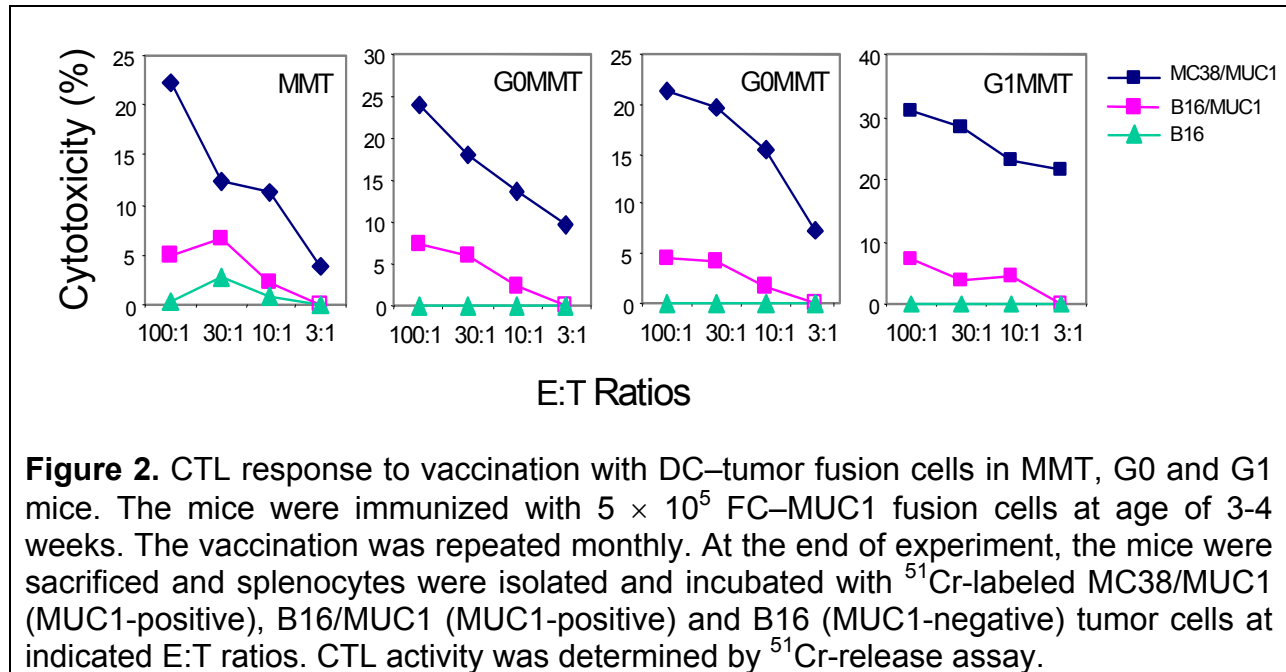


Figure 2. CTL response to vaccination with DC-tumor fusion cells in MMT, G0 and G1 mice. The mice were immunized with 5×10^5 FC-MUC1 fusion cells at age of 3-4 weeks. The vaccination was repeated monthly. At the end of experiment, the mice were sacrificed and splenocytes were isolated and incubated with ^{51}Cr -labeled MC38/MUC1 (MUC1-positive), B16/MUC1 (MUC1-positive) and B16 (MUC1-negative) tumor cells at indicated E:T ratios. CTL activity was determined by ^{51}Cr -release assay.

KEY RESEARCH ACCOMPLISHMENTS:

1. Generation of G0, G1 and G2 mTERC^{-/-}MMT mice.
2. Slightly inhibition of tumor development was observed in G1 mTERC^{-/-}MMT.
3. Comparable induction of CTL activity in MMT, G0 and G1 mTERC^{-/-}MMT mice. The tumor development in G1 mTERC^{-/-}MMT mice immunized with FC/MUC1 was further delayed although the sample is too small to do statistical analysis.

REPORTABLE OUTCOMES:

ABSTRACT

1. William Song, Chunlei Liu and Jianlin Gong. Role of telomerase and telomere maintenance in the development of mammary carcinomas. Conference Proceedings by AACR, A27, November 29, 2006

CONCLUSIONS:

1. We have successfully generated G0, G1 and G2 mTERC^{-/-}MMT mice.
2. Telomerase deficiency has no detrimental effect in the induction of CTL in G1 mTERC^{-/-}MMT mice.

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